

# Online Research @ Cardiff

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: <http://orca.cf.ac.uk/117274/>

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Evans, D. Gareth, Howell, Sacha J., Frayling, Ian M. and Peltonen, Juha 2018. Gene panel testing for breast cancer should not be used to confirm syndromic gene associations. *npj Genomic Medicine* 3 (1) , 32. 10.1038/s41525-018-0071-6 file

Publishers page: <http://dx.doi.org/10.1038/s41525-018-0071-6> <<http://dx.doi.org/10.1038/s41525-018-0071-6>>

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See <http://orca.cf.ac.uk/policies.html> for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.



**Gene panel testing for breast cancer should not be used to confirm associations with syndromic genes in particular for *NF1* pathogenic variants**

**Running Head: Panel testing for syndromic gene cancer associations**

**D. Gareth Evans MD,<sup>1,2,3</sup> Sacha J Howell PhD,<sup>3,4</sup> Ian M. Frayling MD PhD,<sup>5,6</sup> Juha Peltonen MD, PhD<sup>7</sup>**

<sup>1</sup>Manchester Centre for Genomic Medicine, Central Manchester University Hospitals NHS Foundation Trust, Manchester, M13 9WL, UK.

<sup>2</sup>Division of Evolution and Genomic Sciences, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester Academic Health Science Centre, Manchester, UK.

<sup>3</sup>The Christie NHS Foundation Trust, Wilmslow Road, Manchester, M20 4BX, UK.

<sup>4</sup>Division of Cancer Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester Academic Health Science Centre, Manchester, UK

<sup>5</sup>All-Wales Medical Genetics Service, Cardiff & Vale University Health Board, University Hospital of Wales, Cardiff, CF14 4XW, UK

<sup>6</sup>Institute of Medical Genetics, Division of Cancer & Genetics, School of Medicine, Cardiff University, Cardiff, CF14 4XN, UK

<sup>7</sup>Department of Cell Biology and Anatomy, Institute of Biomedicine, University of Turku, Turku, Finland

Correspondence: Prof DG Evans, Department of Genetic Medicine, Manchester Academic Health Sciences Centre (MAHSC), St Mary's Hospital, University of Manchester, Manchester M13 9WL Email: [gareth.evans@mft.nhs.uk](mailto:gareth.evans@mft.nhs.uk)  
Tel: +44 (0)161 276 6506; Fax: +44 (0)161 276 6145  
**Conflict of interest:** None to declare

Word count 1105

## Comment (words 1125)

In recent years there has been an explosion in the use of multi-gene panels to test for cancer predisposition often utilising large panels across many tumour types. More recently the results of these tests have been used as a form of case control study to assess genes for cancer associations, especially with breast cancer. Case control analysis is arguably the most informative method to identify gene-cancer associations as it also provides confirmation of the level of any increased risk. Ideally the 'cases' should be derived from a truly unselected series of individuals with the relevant cancer. Similarly the controls should come from the same population, and can be either true 'population' controls from representative, unselected and ideally age matched individuals, or 'super' controls (older/age matched individuals known not to have the malignancy being investigated). Particularly for rare diseases (present in less than 1 in 2000 individuals), very large case control series are required to confirm moderate risk elevations of only 2-3 fold. Whilst traditional methods such as positional cloning from family linkage were used for identifying the *BRCA1* and *BRCA2* genes in 1994 and 1995, most other breast cancer predisposition genes were identified as causal from candidate gene approaches using case control series often enhanced by using familial samples. However, even these have identified potentially spurious associations, because analyses of breast/ovarian cancer families has identified a real association with 'ovarian' cancer, but a potentially false association with breast cancer<sup>1,2</sup>. In particular, initial breast cancer associations with *RAD51C*, *RAD51D* and *BRIP1* were later called into question with breast cancer specific analysis<sup>1,2</sup>. Hence, these three genes are not on the UK's National Health Service breast cancer panel,<sup>3</sup> but they do appear on most commercial 'breast cancer' specific panels. Other DNA repair gene associations, such as for *ATM*, *CHEK2* and *PALB2*, have been well validated in multiple cohort studies and added to breast cancer specific gene panels.<sup>3</sup> In contrast to the aforementioned genes which have no recognisable syndromic phenotype for an individual, the phenotypes from germline pathogenic variants in *NF1*, *PTEN* and *STK11* (table 1) are usually easily recognisable in single individuals and there are well validated diagnostic criteria which allow a clinical diagnosis without the need for molecular confirmation.<sup>4,5</sup>

Four recent articles based on multi-gene panel testing, published in high impact oncology journals, have nonetheless concluded that there are no associations between pathogenic variants in these three syndromic genes (*NF1*, *PTEN* and *STK11*) and breast cancer risk.<sup>6-9</sup> Whilst these analyses have identified potential new gene associations, the negative results concerning syndromic associations should be tempered. In the first of their three articles Ambry's 21 panel gene test<sup>6</sup> was evaluated in 41,611 consecutively tested white women with breast cancer. In the second<sup>7</sup> 9,639 patients with breast cancer were assessed, whilst the third assessed the risk of triple negative breast cancer in 8,753 women. Whilst two studies used control data from the ExAc database<sup>6,8</sup> the second used a combination of controls tested for non-cancer indications.<sup>7</sup> The larger initial study<sup>6</sup> identified *NF1* gene variants in 0.1% compared to 0.11% in ExAc controls. No control frequency was provided for the second study although a frequency of around 0.15% was said to be non-significant<sup>8</sup>. The third study also found a frequency of 0.15% in triple negative breast cancer which was also non-significant.<sup>8</sup> The first two studies effectively excluded *BRCA1* and *BRCA2* as they confirmed that there had been a high degree of pre-testing for these genes. The first also excluded what they 'termed' 'syndromic' genes including *PTEN*, *CDH1* and *TP53*. However, it is unclear why neurofibromatosis 1, caused by pathogenic variants in the *NF1* gene, was not also excluded as being

syndromic, as it is far more recognisable from patient characteristics than even PTEN hamartoma syndrome.<sup>4</sup>

There are two main flaws in the conclusions that *NF1* is not associated with breast cancer. The first is that panel testing for breast cancer is selective based on family history, age and patient/clinician choice. A clinician who already has an 'explanation' for a breast cancer in a patient with *NF1* is unlikely to send off for a gene panel. Indeed the syndromic learning problems associated with *NF1* may also preclude gainful employment and thus reimbursement for panel testing. The link to breast cancer based on cohort studies is now irrefutable with six studies reporting Odds Ratios of 4-11 fold for *NF1* women aged <50 years of age<sup>10,11</sup>. Furthermore, driver *NF1* pathogenic variants have been identified in the Cancer Genome Atlas and from *NF1* patients are associated with higher tumour grade and Human Epidermal Growth Factor Receptor 2 (HER2) overexpression,<sup>10,12,13</sup> further evidence against a chance association. The link with breast cancer and *NF1* has been established since at least 2007<sup>11</sup>. All of the reported panel tests were performed since March 2012<sup>6-8</sup>, after four of the cohort studies had reported showing a probable causal association with breast cancer and *NF1*<sup>11</sup>. The authors of the Ambry reports clearly admit that there was already preselection for BRCA testing<sup>6-8</sup>. If a clinician wished to test for *NF1* they would use a substantially more sensitive RNA based approach than a panel<sup>14</sup>. A similar concern should also be expressed for the apparent lack of association with *PTEN* in the second and third Ambry studies<sup>7,8</sup> as these were not powered to assess the lower frequency of *PTEN* pathogenic variants and suffer from the same issue of lack of requirement for a panel test when an explanation for breast cancer was already present. The same criticism can be put forward for the absence of *STK11* variants being identified in 2000 familial breast cancer samples tested in the LIFEPOOL study.<sup>9</sup> Peutz-Jeghers disease caused by *STK11* variants is easily identified by peri-oral pigmentation and usually presents symptomatically in early life with multiple intestinal polyps (Table 1).

The second potential flaw is the use of ExAc controls. The very high frequency of apparent pathogenic variants of 1/900 is simply not consistent with the estimates of birth incidence from highly ascertained populations of between 1 in 2,000-2,600<sup>15,16</sup>. Due to early deaths in *NF1* patients prevalence in an adult population is nearer 1 in 3,000-4,500.<sup>15,16</sup> It is therefore unclear whether this high estimate in controls is due to over assessment of pathogenic variants, selection for children to have exomes with *NF1* features or due to some variants being silent clinically.

Whilst panel tests appear to be a useful agnostic test for cancer associations with non-syndromic genes, they are not when considering easily recognisable syndromes, as these create biases in selection against any association and may also contaminate controls. The only way to robustly assess for links with syndromes in a case control study is for ALL patients with breast cancer to be tested on a population basis with appropriate controls tested of a similar age, also without selection.

### **Acknowledgements**

DGE and SJH are supported by the all Manchester NIHR Biomedical Research Centre (IS-BRC-1215-20007). JP is supported by Cancer Society of Finland.

## References

1. Easton, D.F. et al. No evidence that protein truncating variants in BRIP1 are associated with breast cancer risk: implications for gene panel testing. *J Med Genet.* 2016;53(5):298-309
2. Loveday, C. et al. Germline RAD51C mutations confer susceptibility to ovarian cancer. *Nat Genet.* 2012;44(5):475-6
3. Taylor A, Brady AF, Frayling IM on behalf of the UK Cancer Genetics Group (UK-CGG), et al. Consensus for genes to be included on cancer panel tests offered by UK genetics services: guidelines of the UK Cancer Genetics Group *Journal of Medical Genetics* 2018;55:372-377
4. Eng C. PTEN Hamartoma Tumor Syndrome. *GeneReviews*® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2018. 2001 Nov 29 [updated 2016 Jun 2]
5. McGarrity, T.J., Amos, C.I., Baker, M.J. Peutz-Jeghers Syndrome. *GeneReviews*® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2018. 2001 Feb 23 [updated 2016 Jul 14].
6. Couch, F.J. et al. Associations Between Cancer Predisposition Testing Panel Genes and Breast Cancer. *JAMA Oncol.* 2017;3(9):1190-1196
7. Lu, H-M. et al. Association of Breast and Ovarian Cancers With Predisposition Genes Identified by Large-Scale Sequencing *JAMA Oncol.* Published online August 16, 2018. doi:10.1001/jamaoncol.2018.2956
8. Shimelis, H. et al. Triple-Negative Breast Cancer Risk Genes Identified by Multigene Hereditary Cancer Panel Testing. *J Natl Cancer Inst.* 2018 Aug 7. doi: 10.1093/jnci/djy106
9. Thompson, E.R. et al. Panel Testing for Familial Breast Cancer: Calibrating the Tension Between Research and Clinical Care. *J Clin Oncol.* 2016 May 1;34(13):1455-9
10. Uusitalo, E. et al. Breast cancer in neurofibromatosis type 1: overrepresentation of unfavourable prognostic factors. *Br J Cancer* 2017;116(2):211-17. doi: 10.1038/bjc.2016.403
11. Howell, S.J. et al. Increased risk of breast cancer in neurofibromatosis type 1: current insights. *Breast Cancer (Dove Med Press).* 2017 Aug 21;9:531-536
12. Wang, X., et al. Germline and somatic NF1 alterations are linked to increased HER2 expression in breast cancer. *Cancer Prev Res (Phila).* 2018 Aug 13. pii: canprevres.0072.2018. doi: 10.1158/1940-6207.CAPR-18-0072. [Epub ahead of print]
13. Yap, Y.S. et al. Breast cancer in women with neurofibromatosis type 1 (NF1): a comprehensive case series with molecular insights into its aggressive phenotype. *Breast Cancer Res Treat.* 2018 Jun 21. doi: 10.1007/s10549-018-4851-6
14. Evans, D.G., et al. Comprehensive RNA Analysis of the NF1 Gene in Classically Affected NF1 Affected Individuals Meeting NIH Criteria has High Sensitivity and Mutation Negative Testing is Reassuring in Isolated Cases With Pigmentary Features Only. *EBioMedicine.* 2016 May;7:212-20.
15. Evans, D.G. et al. Birth incidence and prevalence of tumor-prone syndromes: estimates from a UK family genetic register service. *Am J Med Genet A.* 2010;152A(2):327-32
16. Kallionpää, R.A. et al. Prevalence of neurofibromatosis type 1 in the Finnish population. *Genet Med.* 2017 Dec 7. doi: 10.1038/gim.2017.215. [Epub ahead of print]

**Table 1:** Syndromic genes identifiable from clinical features in a single woman that increase breast cancer risk from cohort studies

Gene	Syndrome	Birth incidence	Clinical features	Breast cancer risk to 50 yrs/lifetime	Other malignancy risk
<i>NF1</i>	Neurofibromatosis 1	1 in 2,000-2,500	<i>Café au lait</i> Cutaneous neurofibromas Iris Lisch nodules	10%/20%	Malignant peripheral nerve sheath tumor, glioma
<i>PTEN</i>	PTEN hamartoma syndrome (Cowden) <sup>3</sup>	1 in 100,000-200,000	Macrocephaly, mucocutaneous lesions (eg Trichilemmomas)	50%/85%	Thyroid, endometrial
<i>STK11</i>	Peutz-Jeghers <sup>4</sup>	1:25,000 to 1:280,000	Peri-oral pigmentation, hamartomatous bowel polyps	Nk/37-55%	Colorectal, stomach, small bowel, ovary, cervix, pancreas, testes